Biomaterial Interfaces Division
Room 12 - Session BI+AS+SA-ThM

Characterisation of Biological and Biomaterial Surfaces

Moderators: Daniel Graham, University of Washington, Tobias Weidner, Aarhus University, Denmark

8:00am **BI+AS+SA-ThM-1 Lipid Involvement in the Regenerative Processes of *Dugesia dorotocephala* - A GCIB ToF-SIMS Imaging Study*, Tina Angerrer,*** *M Taylor, D Graham, L Gamble,* University of Washington

Dugesia dorotocephala are planaria belonging to the class of Turbellaria, or non-parasitic flat worms. They are best known for their fascinating regenerative abilities, which allow them to be cut into more than 200 pieces, each piece missing essential parts necessary for the worms' survival and each re-growing a new flatworm. This level of reorganization involves a complex interplay of a wide range of molecules that varies spatially and temporally but is still poorly understood.

Recently the involvement of peptides and proteins in the process of regrowing the head and developing a new central nervous system has been studied by Sweedler et al.[1] using MALDI imaging. MALDI, in contrast to TOF-SIMS imaging, is capable of studying the distributions of peptides in tissue but spatial resolution is limited and molecules of interest have to be partially predetermined by the choice of matrix.

Using the J105-3D Chemical Imager, (Ionoptika Ltd) equipped with a 40 keV gas cluster ion beam (GCIB), molecules with sizes up to 2000 Da can be localized at a cellular scale, with spatial resolutions better than 3 µm.[2] Since ToF-SIMS is a label free technique, it can be used in an untargeted discovery approach which, in biological samples, is mainly used to study lipid distributions.

Lipids are a diverse group of molecules fulfilling numerous functions such as energy storage and cell signaling, however lipid and fatty acid data for Dugesia in general is very limited and their localizations completely unknown.[3] Our studies were targeted at establishing a full body lipid profile for the different organ systems present in Dugesia as well as monitoring their changes due to stem cell migration during head regrowth and eye/CNS regeneration.

Dugesia flatworms were sectioned on a cryomicrotome at -20 °C and slices were placed on ITO coated glass. After preparation samples were immediately taken to the lab for analysis. Sample preparation and transport time was kept to less than 2 hours to minimize lipid degradation. After SIMS analysis, optical images were acquired in order to facilitate identification of structures seen within the worms. To deal with the increased spectral and spatial complexity provided by our improved instrumental capabilities, imaging PCA was used to "untangle" the data. In this presentation we will present the results of our studies showing the unique lipid distributions throughout Dugesia cross sections and discuss their relevance.

[1] T. H. Ong, *et al.*, J Biol Chem 2016, 291, 8109-8120.

[2] T. B. Angerer, *et al.*, Int J Mass Spectrom 2015, 377, 591-598.

[3] F. Meyer, *et al.*, Biochim Biophys Acta 1970, 210, 257-&.

8:20am **BI+AS+SA-ThM-2 Can ToF-SIMS Imaging Explain Biology?*, Lara Gamble,*** *D Graham,* University of Washington

Imaging time-of-flight mass spectrometry (ToF-SIMS) can provide images of cells and tissues with chemical and molecular specificity. These chemically specific images could revolutionize our understanding of biological processes such as the role of changes in tumor metabolism affecting the response to chemotherapy is under scrutiny. Regions of interest (ROIs) of the tumor can be utilized to compare similar regions from different tissue samples. PCA analysis of ToF-SIMS image data reveals the differences in chemistries between the regions. These results help to identify links between the chemical composition within and around tumors and the changes of these tumors as a response to the treatment. However, often the presentation of ToF-SIMS results might not be in the best format to gain the interest of non-SIMS scientists. Different data processing and data presentation format from clinical trial tissue samples and other tissue samples analyzed with ToF-SIMS will be presented. Additional validation of data interpretation from different techniques will be discussed.

8:40am **BI+AS+SA-ThM-3 Applications of XPS for Novel Biomaterial Systems*, Jonathan Counsell,*** *S Coultas, C Blomfield,* Kratos Analytical Limited, UK*; C Moffitt,* Kratos Analytical*; S Hutton,* Kratos Analytical Limited, UK, United Kingdom of Great Britain and Northern Ireland **INVITED**

XPS is widely used in the field of biomaterials yielding quantitative elemental and chemical state information [1]. It is possible to identify changes in functional groups present both on the surface and, combined with depth profiling, within the bulk of a biomaterial.

Here we will discuss the latest advancements in XPS as applied to a range of biomaterial systems and examine new possibilities beyond routine spectroscopic analysis. Non-destructive depth profiling of the near surface region is applied to ultra-thin films examining growth modes and film closure mechanisms. With the dual Al/Ag monochromated sources it is possible to vary information depth for relative comparisons on the nature of the uppermost layers. New developments in cluster ion sources now allow soft biomaterials to be depth profiled. Accurate analysis of interfacial chemistry is possible without ion beam damage. XP Imaging will also be discussed for systems exhibiting surface inhomogeneity. Quantitative images yield useful additional information over conventional microscopies. Discussions will concentrate on both model systems and real life applications highlighting the latest possibilities of XPS for this growing field.

[1] Donald R. Miller and Nikolaos A. Peppas, Journal of Macromolecular Science, Part C Vol. 26 , Iss. 1,1986

9:20am **BI+AS+SA-ThM-5 Surface Characterization of Polymer Scaffolds: Understanding Surface Modification and Biological Interactions*, Michael Taylor,*** University of Washington*; M Hawker, M Mann,* Colorado State University*; G Hammer,* University of Washington*; E Fisher,* Colorado State University*; D Graham, L Gamble,* University of Washington

Biopolymers show increasing usage in medical device technologies including joint replacement, stents and tissue engineered supports. (polymer scaffolds). Barriers to successful use of biopolymer usage for medical devices can include ineffective interaction of biological systems with the biopolymer and biofilm formation. Historically, developing medical devices with antibacterial properties have involved inclusion of silver or copper dopants as they facilitate bacterial membrane rupture. Bacterio-static coatings provide an alternative approach by generating a hydrophobic surface that prevents colonisation by reversible adhesion via van der Waals forces prior to anchoring strongly with adhesion structures such as pili. Plasma enhanced chemical vapor deposition (PECVD) is a cheap yet powerful method of introducing chemical functionalities to surfaces as the low temperature high energy process may be used to couple a variety of monomers to biomaterial surfaces. Previous evidence provided by Fisher and coworkers showed that PECVD may be utilised to produce antifouling coatings by modifying polycaprolactone (PCL) with fluorinated organic compounds[1], however the porous morphology of scaffolds required for vascularisation also provides multiple points of attachment for the critical first step in biofilm formation. It is therefore necessary determine the effectiveness of PECVD throughout the scaffold. For this we employ time-of-flight secondary ion mass spectrometry (ToF-SIMS) and X-ray photoelectron spectroscopy (XPS) to analyse the surface modification of porous polymer scaffolds.

ToF-SIMS imaging coupled with X-ray photoelectron spectroscopy (XPS) provides a powerful combination of high resolution imaging and elemental quantitative analysis that allows a detailed analysis of the surface. Herein we apply this combination of analysis methods for the determination and quantification of fluorocarbon distribution across a PCL scaffold modified with octafluoropropane by PECVD, determining that a treatment time of 20 minutes Introduces a homogeneous distribution of fluorocarbon film throughout the construct cross section whereas lower treatment times produces a gradient distribution of fluorocarbon, as measured via CF^+ and CF_3^+ signals

(1) Hawker, M. J.; Pegalajar-jurado, A.; Fisher, E. R. Conformal Encapsulation of Three-Dimensional, Bioresorbable Polymeric Sca Ff Olds Using Plasma-Enhanced Chemical Vapor Deposition. **2014**.

9:40am **BI+AS+SA-ThM-6 Seawater Bacteria on Technical Surfaces: Lateral and Vertical Adhesion Forces and Nanomechanical Properties***, N Davoudi, K Huttenlochner,* University of Kaiserslautern, Department of Physics and Research Center Optimas, Germany*; C Schlegel, M Huster,* University of Kaiserslautern, Institute of Bioprocess Engineering, Germany*;* ***Christine Müller-Renno****,* University of Kaiserslautern, Department of Physics and Research Center Optimas, Germany*; R Ulber,* University of Kaiserslautern, Institute of Bioprocess Engineering, Germany*; C Ziegler,* University of Kaiserslautern, Department of Physics and Research Center Optimas, Germany, Germany

Biofilms are often unwanted, but can also be utilized in biofilm reactors. In such a reactor different forces act on the cells: lateral forces by flow, forces perpendicular to the interface which dominate the first contact and the biofilm formation, and forces on the cell-wall by turgor pressure which influence the viability of the cells. The interplay of these forces plays a major role in the establishment of a biofilm.

Here, we report on the seawater bacterium *Paracoccus seriniphilus* on titanium and glass. Microstructured titanium is our substrate of choice in the reactor. We hence have to understand the influence of wettability, roughness, defined structures, and environmental conditions such as pH and ionic strength on the viability as well as the bacterial attachment and detachment.

In a first set of experiments, the turgor pressure of the bacteria was determined as a function of pH and salinity by measuring force-distance curves with a scanning force microscope (SFM). As a seawater bacterium, *P. seriniphilus* can easily adapt to saline conditions and can survive at NaCl concentrations up to 100 gL^{-1}. Depending on the ionic strength the turgor pressure and thus the elasticity and size of the cell changes. *P. seriniphilus* has its optimum pH at 7, but at pH 4 the results point to an active adaption mechanism to acidic conditions. The results at pH 11 show that *P. seriniphilus* cannot adapt to alkaline conditions.

As next step the vertical adhesion forces of a single bacterium were measured as a function of pH, ionic strength, and substrate. The adhesion force of one single cell decreases from pH 4 to pH 9. As a function of the ionic strength, the adhesion forces increase with increasing salt concentration with a pronounced spike (higher adhesion forces) at 0.9 % NaCl. All adhesion force changes completely correlate with the electrostatics as determined by zetapotential measurements. A conditioning film of growth medium strongly decreases the attachment forces. Thus the first bacterial layer should grow without medium at pH 4.

In a last step, the lateral detachment forces of the bacteria were measured. There is a clear correlation between the applied force and the number of moved bacteria, but the detachment forces vary for the individual bacteria. For small lateral forces (0.5 nN), the wettability of the substrate seems to control the detachment process. For higher lateral forces (2-3 nN), the effect of the wettability gets lost and the roughness of the samples controls the cell detachment. These detachment forces are in the same range or higher than the shear forces applied by the fluid flow.

11:00am **BI+AS+SA-ThM-10 AVS 2017 Peter Mark Memorial Award Lecture: A Combined Experimental–Simulation Approach for Unraveling Hydrophobic Interactions at the Molecular Scale***, P Stock,* MPI for Iron Research, Germany*; J Monroe,* UC Santa Barbara*; T Utzig,* MPI for Iron Research, Germany*; D Smith, S Shell,* UC Santa Barbara*;* ***Markus Valtiner***[1]*,* TU Bergakademie Freiberg, Germany **INVITED**

Interactions between hydrophobic moieties steer ubiquitous processes in aqueous media, including the self-organization of biologic matter. Recent decades have seen tremendous progress in understanding these for macroscopic hydrophobic interfaces. Yet, it is still a challenge to experimentally measure hydrophobic interactions (HIs) at the single-molecule scale and thus to compare with theory.

Here, I will present a combined experimental–simulation approach to directly measure and quantify the sequence dependence and additivity of HIs in peptide systems at the single-molecule scale. We combined dynamic single-molecule force spectroscopy on model peptides with fully atomistic, both equilibrium and nonequilibrium, molecular dynamics (MD) simulations of the same systems. Specifically, we mutate a flexible $(GS)_5$ peptide scaffold with increasing numbers of hydrophobic leucine monomers and measure the peptides' desorption from hydrophobic self-assembled monolayer surfaces. Based on the analysis of nonequilibrium work-trajectories, we measure an interaction free energy that scales linearly with 3.0–3.4 k_BT per leucine. In good agreement, simulations indicate a similar trend with 2.1 k_BT per leucine, while also providing a detailed molecular view into HIs.

Our approach potentially provides a roadmap for directly extracting qualitative and quantitative single-molecule interactions at solid/liquid interfaces in a wide range of fields, including interactions at biointerfaces and adhesive interactions in industrial applications. In this context, I will finally discuss in detail how single molecule unbinding energy landscapes can be utilized to predict scenarios where a large number of molecules simultaneously interact, giving rise to adhesive failure under corrosive and wet conditions.

[1] S. Raman et al. *in Nature Communications***,** 5**(2014)**, 5539.

[2] T. Utzig et al. *in Langmuir*, 31(9) **(2015)**, 2722.

[3] T. Utzig, P. Stock et al. *in Angewandte Intl.***(2016)**.

[4] P. Stock et al. in *ACS Nano***(2017)**, 11 (3), 2586.

11:40am **BI+AS+SA-ThM-12 Quantitative Characterization of Bacterial Cells in Solution and on Surfaces***, C Sousa, K Jankowska, L Parga Basanta, I Pinto,* ***Dmitri Petrovykh****,* International Iberian Nanotechnology Laboratory, Portugal

Physicochemical properties of bacterial cells make them challenging subjects for methods typically used to characterize micro- and nanoparticles. Even for conceptually simple parameters, such as size and concentration, direct characterization of live bacteria (and their agglomerates) in solution is far from trivial because bacterial cells are soft and often anisotropic particles with sizes of not more than a few microns. Low contrast, in terms of optical and electronic properties, between bacteria and their aqueous environment complicates any attempted direct measurements in solution. Comparing bacterial cells to non-biological micro- or nanoparticles, whether in the context of mixed samples or calibration measurements, further compounds the complexity of characterizing these systems.

We are using *Staphylococcus aureus* (*S. aureus*) bacteria as a model system for quantitative characterization of bacterial cells. For systematic measurements, *S. aureus* bacteria offer the advantages of nearly spherical shape and of robust viability under a wide range of experimental conditions and treatments. The approximately one micron diameter of live *S. aureus* cells also makes them representative of the sensitivity and resolution challenges encountered in the characterization of bacterial cells. In microscopy, for example, the apparent size of individual *S. aureus* bacteria changes dramatically as they are prepared for measurements with increased spatial resolution: from confocal optical microscopy, to environmental scanning electron microscopy (SEM), to SEM in vacuum.

The objective of our work is to develop and validate a set of complementary techniques that can be used to characterize live bacterial cells. We will describe the use of nanoporous membranes with *S. aureus* suspensions and commonly overlooked effects of centrifugation, mechanical agitation, and other typical sample preparation procedures on the apparent distribution and properties of particles in biological samples. The forced contact of bacteria with these membranes during filtering also suggests their use as model systems for investigating the interactions of bacteria with surfaces having different chemistries and/or morphological features.

12:00pm **BI+AS+SA-ThM-13** ***In Situ*** **Multimodal Imaging of Microbial Communities***, Xiao-Ying Yu,* Pacific Northwest National Laboratory

We developed a vacuum compatible microfluidic interface, System for Analysis at the Liquid Vacuum Interface (SALVI), to enable direct observations of liquid surfaces and liquid-solid interactions using time-of-flight secondary ion mass spectrometry (ToF-SIMS) and a variety of spectroscopy and microscopy characterization techniques. SALVI was recently applied to investigate biological interfaces in living biofilms and co-cultured microbial communities. In this talk, two case studies will be presented using in situ liquid ToF-SIMS, light microscopy, and fluorescence microscopy. In the first case study, *Shewanella* wild type and mutant were both exposed to environmental stressors such as toxic heavy metal ions (i.e., Cr (VI)) and silver nanoparticles. The response of biofilm and its extracellular polymeric substance (EPS) to the environmental perturbation was investigated using in situ liquid SIMS coupled with structured illumination microscopy (SIM). In the second case, a more complex microbial communities consisting of syntrophic *Geobacter metallireducens* and *Geobacter sulfurreducens* was investigated. Electron donor and electron acceptor in this co-cultured microbial system were characterized first using the more traditional SIMS dry biological sample preparation approach followed by in situ liquid SIMS and confocal laser scanning

[1] **Peter Mark Memorial Award Winner**

microscopy (CLSM). The electron transfer between the two species was probed dynamically using the electrochemical SALVI. Correlative imaging is employed to achieve a more holistic view of complexed microbial systems across different space scales. Our results demonstrate that interfacial chemistry involving living microbial systems can be studied from the bottom up based on microfluidics, potentially providing more important understanding in system biology.

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